



INTERNATIONAL PRELIMINARY EXAMINATION REPORT  
(PCT Article 36 and Rule 70)

Applicant's or agent's file reference P044829PCT BSW/do	<b>FOR FURTHER ACTION</b> See Notification of Transmittal of International Preliminary Examination Report (Form PCT/PEAA16)	
International application No. PCT/NL 03/00049	International filing date (day/month/year) 23.01.2003	Priority date (day/month/year) 23.01.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/61		
Applicant ROYAL NEDALCO B.V. et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 4 sheets, including this cover sheet.
- ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).
- These annexes consist of a total of 3 sheets.

3. This report contains indications relating to the following items:
- I ☒ Basis of the opinion
  - II ☐ Priority
  - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
  - IV ☐ Lack of unity of invention
  - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
  - VI ☐ Certain documents cited
  - VII ☐ Certain defects in the international application
  - VIII ☐ Certain observations on the international application

Date of submission of the demand  01.08.2003	Date of completion of this report  26.04.2004
Name and mailing address of the international preliminary examining authority:   European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer  Dumont, E  Telephone No. +49 89 2399-7704  

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. PCT/NL 03/00049

**I. Basis of the report**

1. With regard to the **elements** of the international application (*Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17)*):

**Description, Pages**

1-22 as originally filed

**Claims, Numbers**

1-17 received on 13.04.2004 with letter of 13.04.2004

**Drawings, Sheets**

1/1 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).
3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:
- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT**

International application No. **PCT/NL 03/00049**

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes: Claims	1-17
	No: Claims	
Inventive step (IS)	Yes: Claims	1-17
	No: Claims	
Industrial applicability (IA)	Yes: Claims	1-17
	No: Claims	

2. Citations and explanations

**see separate sheet**

**INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/NL03/00049

Reference is made to the following documents:

- D1: DATABASE EMBL [Online] 3 March 2000 (2000-03-03) OP DEN CAMP H.J.M.: 'Piromyces sp. E2 mRNA for xylose isomerase (xylA gene)' Database accession no. AJ249909 XP002201310
- D3: WO 96 24667 A (PRIMALCO LTD ;SUOMALAINEN IRMA (FI); AHO SIRPA (US); SAARELAINEN R) 15 August 1996 (1996-08-15)
- D5: Harhangi et al., Arch Microbiol. (2003) 180: 134-141 (cited by the Applicant)

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

The subject-matter of claims 1-17, which relates to a eukaryotic host cell transformed with a xylose isomerase having at least 70% sequence identity with the specific xylose isomerase according to SEQ ID NO: 1 isolated from the fungus *Piromyces sp. E2*, is considered novel and inventive with regard to the cited prior art.

In the prior art, expression of prokaryotic xylose isomerases in *S. cerevisiae* did not lead to active xylose isomerase, or did not lead to sufficient activity at physiological temperatures (see e.g. D3, p. 2, § 4; present application, p. 2, l. 26-p. 3, l. 8). D3 reports the isolation of a eukaryotic isomerase from barley, and suggests that the eukaryotic barley enzyme will be more efficiently expressed in yeast than bacterial enzymes, because of the genetic similarities between the eukaryotic plant cell from which the enzyme is derived and the eukaryotic yeast cell in which it is expressed (D3, p. 3, § 1).

The *Piromyces* xylose isomerase disclosed in D1 is more closely related to prokaryotic xylose isomerases than to eukaryotic xylose isomerases (D5, post-published document cited by the applicant). It could therefore not have been derived from the prior art that the xylose isomerase from *Piromyces sp. E2* would be expressed in *S. cerevisiae* and would confer to *S. cerevisiae* the ability to grow on xylose as carbon source.

EPO - DG 1

PCT/NL03/00049

1

13. 04. 2004

Amended Claims

(94)

1. A eukaryotic host cell transformed with a nucleic acid construct comprising a nucleotide sequence encoding a xylose isomerase comprising an amino acid sequence  
5 that has at least 70 % sequence identity with the amino acid sequence of SEQ ID NO: 1, whereby the nucleic acid construct, upon transformation of the host cell, confers to the host cell the ability to grow on xylose as carbon source.
2. A transformed host cell according to claim 1, wherein the host cell is a yeast,  
10 preferably a yeast that belongs to one of the genera: *Saccharomyces*, *Kluyveromyces*, *Candida*, *Pichia*, *Schizosaccharomyces*, *Hansenula*, *Kloeckera*, *Schwanniomyces*, and *Yarrowia*.
3. A transformed host cell according to claim 2, wherein the yeast belongs to one of  
15 the species: *S. cerevisiae*, *S. bulderi*, *S. barnetti*, *S. exiguus*, *S. uvarum*, *S. diastaticus*, *K. lactis*, *K. marxianus*, and *K. fragilis*.
4. A transformed host cell according to claim 1, wherein the host cell is a filamentous fungus, preferably a filamentous fungus that belongs to one of the genera:  
20 *Aspergillus*, *Trichoderma*, *Humicola*, *Acremonium*, *Fusarium*, and *Penicillium*.
5. A transformed host cell according to any one of the preceding claims, whereby the nucleotide sequence encoding a xylose isomerase is operably linked to a promoter that causes sufficient expression of the xylose isomerase in the host cell, to confer to  
25 the host cell the ability to isomerise xylose into xylulose.
6. A transformed host cell according to claim 6, whereby the promoter is insensitive to catabolite repression in the host cell.
7. A transformed host cell according to any one of the preceding claims, whereby  
30 the host cell comprises a genetic modification that result in a characteristic selected from the group consisting of:  
(a) increase transport of xylose into the host cell;

- (b) increased xylulose kinase activity;
- (c) increased flux of the pentose phosphate pathway;
- (d) decreased sensitivity to catabolite repression;
- (e) increased tolerance to ethanol, osmolarity or organic acids; and,
- 5 (f) reduced production of by-products.

8. A transformed host cell according to claim 7, wherein the genetic modification consist of overexpression of endogenous genes, expression of a heterologous genes, or a combination thereof, and whereby the gene is selected from the group consisting of a  
10 gene encoding: a hexose or pentose transporter, an xylulose kinase; an enzyme from the pentose phosphate pathway, a glycolytic enzyme, and an ethanologenic enzymes.

9. A transformed host cell according to claim 7, wherein the genetic modification consist of the inactivation of an endogenous genes, whereby the gene is selected from  
15 the group consisting of a gene encoding a hexose kinase gene, the *Saccharomyces MIG1* and *MIG2* genes and hybridising homologues thereof.

10. A transformed host cell according to any one of the preceding claims, whereby the host cell expresses one or more enzymes that confer to the host cell the ability to  
20 produce lactic acid, acetic acid, succinic acid, amino acids, 1,3-propane-diol, ethylene, glycerol,  $\beta$ -lactam antibiotics and cephalosporins.

11. A transformed host cell according to claim 10, whereby the host cell contains a genetic modification that results in decreased alcohol dehydrogenase activity.  
25

12. A process for producing ethanol, whereby the process comprises the steps of:  
(a) fermenting a medium containing a source of xylose with a transformed host cell as defined in any one of claims 1 - 9, whereby the host cell ferments xylose to ethanol,  
30 and optionally,  
(b) recovery of the ethanol.

13. A process according to claim 12, whereby the medium also contains a source of glucose.

14. A process according to claims 12 or 13, whereby the volumetric ethanol  
5 productivity is at least 0.5 g ethanol per litre per hour.

15. A process according to claims any one of claims 12 - 14, whereby the ethanol yield is at least 50 %.

10 16. A process for producing a fermentation product selected from the group consisting of lactic acid, acetic acid, succinic acid, amino acids, 1,3-propane-diol, ethylene, glycerol,  $\beta$ -lactam antibiotics and cephalosporins, whereby the process comprises the steps of:

- 15 (a) fermenting a medium containing a source of xylose with a transformed host cell as defined in claims 10 or 11, whereby the host cell ferments xylose to the fermentation product, and optionally,  
(b) recovery of the fermentation product.

17. A process according to claim 16, whereby the medium also contains a source of  
20 glucose.